Project #1024777

Title: In Situ Community Control of the Stability of Bioreduced Uranium

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Results To Date: OBJECTIVES:The overall objective of this research is to understand the mechanisms for maintenance of bio-reduced uranium in an aerobic to microaerophylic aquifer under actual field conditions after electron donor addition for biostimulation has ended. Primary Objectives: 1) Determine the relative importance of microbial communities and/or chemical and physical environments mediating uranium reduction/oxidation after cessation of donor addition in an aerobic aquifer. 2) Determine, after cessation of donor addition, the linkages between microbial functions and abiotic processes mediating. Initial Hypotheses: 1) The typical bio-reduced subsurface environments that maintain U(VI) reduction rates after biostimulation contain limited amounts of oxidized iron on mineral surfaces. Therefore, the non sulfate-reducing dissimilatory iron reducing bacteria will move to more conducive areas or be out-competed by more versatile microbes. 2) Microbes capable of sulfate reduction play an important role in the post-treatment maintenance of bio-reduced uranium because these bacteria either directly reduce U(VI) or generate H2S, and/or FeS0.9 which act as oxygen sinks maintaining U(IV) in a reduced state. 3) The presence of bioprecipitated amorphous FeS0.9 in sediments will maintain low U(IV) reoxidation rates under conditions of low biomass, but FeS0.9 by itself is not sufficient to remove U(VI) from groundwater by abiotic reduction. FIELD SCALE EXPERIMENTS: Field-scale electron donor amendment experiments were conducted in 2002, 2003, and 2004 at the Old Rifle Uranium Mill Tailings Remedial Action (UMTRA) site in Rifle, Colorado. The objective of the 2003 experiment (done in collaboration with the U.S. Department of Energy?s UMTRA Groundwater Project) was to test the hypothesis that amendment of increased concentration of electron donor would result in an increased export of electron donor down gradient which in turn would create a larger zone of down-gradient U(VI) bioreduction sustained over a longer time. During the first experiment (2002), ~3 mM acetate was amended to subsurface over a period of 3 months in a 15m x 18m x 2.5m volume comprised of 3 upgradient monitoring wells, 20 injection wells, and 15 down-gradient monitoring wells. After a one-month phase of metal reduction, bioavailable oxidized Fe was consumed near the injection gallery and the dominant terminal electron accepting process became sulfate reduction, rapidly consuming the injected acetate. For the 2003 experiment, we amended sufficient acetate (~10 mM) to consume available sulfate and export acetate down-gradient where bioavailable oxidized Fe was still present. Data from the experiment indicate that acetate was exported further down gradient, resulting in a larger zone of microbial U(VI) reduction than for the 2002 experiment. LONG TERM MICROBIAL RESULTS FROM THE 2003 EXPERIMENT: Up gradient and before acetate injection analysis by PCR of 16S rDNA with DGGE separation and sequencing showed the community to be dominated by beta-proteobacteria (Hydrogenophaga, Dechloromonas, Rhodoferax, Ferribacterium, Rhodocyclus, Methylophilus, Azoarcus),and gamma-Proteobacteria Pseudomonas. Mainly a Gram-negative, heterotrophic, carbon-limited community. The carbon limitation was reflected in the PLFA showing high cyclo/monoenoic PLFA ratios for the samples analyzed. Quinone analysis also suggested strongly a community of facultative-anaerobic microbes living in an oxygenated environment as reflected in a respiratory quinone ratio (ubiquinone/menaquinone ratio) of ~2. It is very likely this community utilized

refractory organics as carbon sources. With biostimulation by acetate injection there was a rapid stimulation of DIRB and SRB and the utilization of oxygen and traces of nitrate coincident with the decrease in groundwater U(VI). Seven to nine months after acetate infusion stopped, increased proportions of sulfide and sulfur oxidizing bacteria were predominant (Sulfuricurvum an epsilon group proteobacteria) plus beta and gamma Proteobacteria. Gram-positive Clostridia, and other SRB appeared and the system maintained the low levels of groundwater U(VI). With these facts in hand we propose a hypothetical model for the processes(s) involved in the maintenance of lowered U(VI) levels in the water. On infusing acetate to the carbon starved community beta-proteobacteria bloom and metabolism quickly utilizes the oxygen in the groundwater and sediments leading to an anaerobic system. This will be reflected in shifts in respiratory quinone profiles and the appearance of plasmalogen lipids. This now anaerobic system contains large surfaces of oxidized Fe(III) forms and is an ideal milieu for rapid Geobacter growth. Geobacter comes to dominate the environment and forms a large active biomass until all the local Fe(III) is reduced. With the loss of reducible iron surfaces some of the wave of Geobacter DIRB swim downgradient to continue utilizing the acetate and exposed Fe(III). Some must lyse as there is a major drop of viable biomass over time. With continued acetate infusion and lysate availability plus sulfate from the sediments and also continual groundwater refreshment, the SRB come to prominence in numbers and diversity generating biomass producing HS- which reacts with iron to form HS- and FeS0.9. Once the acetate infusion is terminated, the biomass decreases by migration and lysis. The lysis products feed the heterotrophic bacteria maintaining the anaerobiosis and allow especially tough Gram-positive bacteria like Clostridia and Desulfotomaculum (+ SRB) to continue to reduce U(VI) so the levels in the groundwater remain low. They produce acid lowering the carbonate and depressing solubilization of U(VI). The large amounts of FeS0.9 and HS- stimulate a complex community of sulfur oxidizing bacteria that use every trace of nitrate or oxygen. With no nitrate or oxygen as terminal electron acceptors for bacteria like Dechloromonas and Geobacter and others they are prevented from reoxidizing U(IV) with oxygen or nitrate as terminal electron acceptors. This model makes some predictions that we should find at long lasting sites where uranium remains stable. There should be high levels of FeS0.9, sulfur oxidizing bacteria, SRB, a somewhat low pH and low carbonate concentration, a carbon, phosphate or nitrogen (or combination) limited community and a low ratio of UQ/MK respiratory quinones. SUPPORT OF ON-GOING FIELD EXPERIMENTS: CBA assisted PNNL in the collection of groundwater samples for routine geochemical analysis. For routine geochemical analysis prior to the 2006 biostimulation experiments, groundwater samples were obtained from the 2005 gallery (B-02, B-05, M-21, M-22, M-23, and M-24), plus M-08 and M-02. Unfiltered samples were retained for in-field analysis of sulfide and Fe(II). Filtered groundwater samples were shipped to PNNL for analysis of cations, anions, and organic carbon. An additional unfiltered sample was preserved with zinc acetate and shipped to PNNL for sulfide analysis. Filtered groundwater samples were also shipped to Helen Vrionis (UMass) for analysis of iron, U(VI), and acetate. CBA assisted PNNL in the sampling of multilevel passive samplers located in monitoring wells B-05, M-08, M-22, and M-23. Water samples from the passive cells were filtered and shipped to PNNL for analysis of organic carbon, cations, anions, and iron. An unfiltered sample was also shipped for analysis of sulfide. METHODS In-well Sediment Incubators: Direct estimation of reoxidation rates is difficult under field conditions. We have designed and fabricated a prototype in-well sediment incubator (IS) for use in conducting a series of in situ experiments that enable direct measurement of U(IV) removal rates from pre-reduced sediments with specific microbial and mineralogical amendments. By comparing U(IV) loss rates with different DIRB and SRB populations we can clearly determine the relative impact of sulfate reducers vs. Fe reducers. It makes possible

assessing in situ conditions during the experiment and to observe reoxidation (or bioreduction) end points after the field experiment is completed without drilling. Finally, the production of in-well sediment incubators is relatively inexpensive and could become an alternative to field-scale electron donor amendment experiments as a means of assessing site response to bioremediation and long-term stability of both biostimulated and naturally bioattenuated sites. Native Rifle aquifer background sediments have been used as the starting material for all in-well sediment incubator (ISI) studies conducted at the site. Sieved background sediment was amended with an electron donor solution and incubated in an anaerobic glovebox to permit generation of a reducing environment. Reduced sediments were sealed, shipped to the site, and loaded into the ISI in the field. Subsamples of the background sediments and reduced sediments were retained for analysis. The ISI was deployed in background well B-02 for a period of three months (December 2005 through March 2006). Following deployment, the ISI was recovered from B-02 and sediment samples were obtained for mineralogical and microbial characterization. The ISI was loaded with background sediment and deployed in down gradient well MW-22 for approximately 3 months (February through May 2006). The ISI was recovered and sediment samples were obtained for analysis. The ISI was then loaded with background sediment and was redeployed in background well B-02. Q-PCR: Real-time PCR focused on enumeration of bacteria(6) delta-Proteobacteria(5) as an indicator of IRB and SRB, Geobacter sp.(5) , Anaeromyxobacter (4) as described. Q-PCR with DSR1F-DSR4R9(8)was used to enumerate dissimilatory sulfite reductase for sulfate reducing bacteria. DGGE and Sequence Analysis: DGGE was performed using a D-Code 16/16 cm gel system.(1). Prominent bands were excised, sequenced and identified with the Sequence Match function of the Ribosomal Database Project (2) and the BLASTN function of the NCBI. Phylogenetic analysis was by SEQBOOT, DNAdist, and NEIGHBOR algorithms within the PHYLIP 3.65 analysis package (3). PRELIMINARY RESULTS - IN-WELL SEDIMENT INCUBATOR: Background Sediments: The DGGE profile of the background sediment was a typical background of faint smear of PCR products with 4-5 discernable bands. The nucleotide sequences of select, discernable bands were determined to establish potentially important members of the bacterial community in the background sediment. The sequences of the selected dominant bands were most closely related to an uncultured Chloroflexi bacterium, Arthrobacter uratoxydans, and an uncultured Terrabacter species. The green non-sulfur bacteria (Chloroflexi) and Actinobacteria (Terrabacter sp.) were predominant members of the bacterial community, consistent with previous results for background samples at the site (7). Firmicutes sequences have also been recovered from Rifle background sediment samples previously (7). Geobacteraceae-like 16S rDNA sequences were not recovered from selected bands in the DGGE fingerprint, however, Geobacter species were detected in low copy number (102 copies/g) by Q-PCR. Likewise, delta-proteobacteria, often used as an indicator of iron-reducing and sulfate-reducing bacteria(5) were detected in low copy numbers (<104 copies/g) in Q-PCR assays. Reduced Sediments prior to ISI deployment: The DGGE fingerprint of reduced sediment samples prior to ISI loading and deployment was a smear of PCR products with 5-7 intense bands. Selected bands suggested enrichment of Firmicutes (mainly Clostridiaceae and Bacillaceae) following electron donor addition in the laboratory. An increase in Firmicutes has also been observed at one location (M-08) following electron donor injection (7). 16S rDNA of delta-proteobacteria increased (p=0.052) following electron donor addition. A significant increase in Geobacter sp. was not observed, but high variability in copy numbers from initial subsample replicates may have masked any increase. Dissimilatory sulfite reductase (DSR) genes were detected, but DSR copy numbers in reduced sediment samples were not significantly greater than in background sediments. Reduced Sediments deployed in Background Well B-02: The ISI containing the reduced

sediments was incubated in background well B-02 for approximately 3 months. Following incubation, the ISI was recovered and sediment samples were obtained for PLFA and DNA analysis. The DGGE fingerprint was a dark background smear with 10-15 dominant bands. Of the eleven bands excised for sequencing, six were most closely related to Firmicutes (Clostridiaceae and Bacillaceae) and four most closely resembled 16S rRNA genes of Bacteroidetes. The presence of members of the Bacteroidetes phylum were not observed in reduced sediment samples prior to deployment suggesting shifts during incubation in B-02. No significant changes were observed in Q-PCR enumeration of specific 16S rRNA and functional genes; Delta-proteobacteria and Geobacter sp. were detected on the order of 104 copies/g in both reduced sediments and ISI samples after incubation in B-02. The DGGE and Q-PCR results suggest that the main changes in the bacterial community composition resulted from substrate addition and only minor changes followed deployment of the reduced sediment in B-02. The background sediment community, based on DGGE and sequencing of major bands, was dominated by green non-sulfur bacteria and Actinobacteria. Substrate addition to promote reducing conditions appeared to cause a shift in the microbial community composition and enrichment of Firmicutes similar to M-08 in field experiments (7). Furthermore, increases in delta-proteobacterial cell densities and a potential increase in Geobacter sp. were noted following electron donor addition, although Bacteria cell density remained relatively constant (108 copies/g). Following a 3 month incubation period in the micro aerobic background well, some enrichment of Bacteroidetes was observed, but Firmicutes appeared to remain dominant members of the microbial community. No significant changes in cell densities of known iron reducing and sulfate reducing bacteria were observed by Q-PCR. 1. Chang, Y. J.,et.al,1999. J. Microbiol. Meth.,40:19-31. 2. Cole, J. R.,et.al, 2005. Nucleic Acids Res 33:D294-D296. 3. Felsenstein, J. 2005. PHYLIP (Phylogeny Inference Package), 3.6 ed. Department of Genome Sciences, University of WA, Seattle, WA. 4. Petrie, L., et.al, 2003.AEM, 69:7467-79. 5. Stults, J. R.,et.al, 2001.AEM, 67:2781-9. 6. Suzuki, M. T., et.al, 2000.AEM, 66:4605-14. 7. Vrionis, H. A., et.al. 2005.AEM, 71:6308-6318. 8. Wagner, M.,et.al, 1998. J Bacteriol, 180:2975-82.

Deliverables: Published Abstracts DOE-ERSD PI Meeting 2006, Paper in review:. David C. White, Philip E. Long, James P. McKinley, Aaron D. Peacock, Brett R. Baldwin, David B. Hedrick, Amanda N. Smithgall, Margaret Gan, Kelly Nevin, and Derek Lovley. Investigating in situ microbial community control of reduced Uranium. Department of Energy-Environmental Remediation Sciences Division PI Meeting. April 3-5, 2006. Warrenton, VA. Brett R. Baldwin, James P. McKinley, Aaron D. Peacock, Melora Park, Dora Ogles, Jonathan D. Istok, Charles T. Resch, and David C. White. Multilevel Samplers to Assess Microbial Community Response to Biostimulation. AGU Joint Assembly. May 23-26, 2006. Baltimore, MD. Baldwin, B.R., A.D. Peacock, M. Park, D.M. Ogles, J.D. Istok, J.P. McKinley, C.T. Resch, and D.C. White. Multilevel Samplers to Assess Microbial Community Response to Biostimulation. (submitted to Ground Water). White D. C., P. E. Long, and S. M. Pfiffner. Microbial Processes by Lipid Anlysis at a Subsurface Uranium Bioimmobilization Site Utilizing 13-C-Acetate. ASM General Meeting. May 21-25, 2006. Orlando, FL.